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No evidence for novel weapons: biochemical recognition modulates early ontogenetic processes in native species and invasive acacias

Florencia A. Yannelli · Ana Novoa · Paula Lorenzo · Jonatan Rodríguez · Johannes J. Le Roux

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Abstract The Novel Weapons Hypothesis postulates that the release of allelochemicals by alien plants can inhibit the growth of evolutionary naïve native plants. On the other hand, when species share a recent evolutionary history, recognition of phytochemicals from neighboring plants can have adaptive value by providing cues to signal suitable conditions conducive to establishment. This has been termed the Biochemical Recognition Hypothesis. We explored these two hypotheses by conducting germination experiments in South Africa and Spain and a growth experiment in South Africa, using invasive Australian acacias and native species from each region. The experiments exposed seeds of the selected recipient species to leachates collected under acacias, nearby uninvaded vegetation, or distilled water. We then measured total germination, and above and below ground biomass in the growth experiment. Our results did not support the Novel Weapons Hypothesis, but instead we found some leachates collected under acacias and uninvaded areas to stimulate the germination and early growth of some of our selected acacias and native species. Such

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F. A. Yannelli (✉) · A. Novoa · J. J. Le Roux
Department of Botany and Zoology, Centre for Invasion Biology, Stellenbosch University, Matieland 7602, South Africa
e-mail: florenciayannelli@gmail.com

A. Novoa
Department of Invasion Ecology, Institute of Botany, Academy of Sciences of the Czech Republic, 252 43 Průhonice, Czech Republic

A. Novoa
South African National Biodiversity Institute, Kirstenbosch Research Centre, Claremont, South Africa

P. Lorenzo
Department of Life Sciences, Centre for Functional Ecology - Science for People and the Planet (CFE), University of Coimbra, Calçada Martim de Freitas, 3000-456 Coimbra, Portugal

J. Rodríguez
Plant Ecophysiology Group, Department of Plant Biology and Soil Science, University of Vigo, 36310 Vigo, Spain

J. Rodríguez
ECOEVO Lab, E. E. Forestal, University of Vigo, 36005 Pontevedra, Spain

J. J. Le Roux
Department of Biological Sciences, Macquarie University, Sydney 2109, Australia

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effects occurred both at the intra- and interspecific level. In general, interspecific stimulatory effects between invasive acacias occurred irrespective of whether they had overlapping native ranges in Australia. We also found leachates from uninvaded areas in South Africa to have stimulatory effects on one invasive acacia and one native species. Hence, our results support the Biochemical Recognition Hypothesis, suggesting that chemically-induced signals may facilitate acacia establishment in sites that have already been transformed by acacias.

Keywords Allelopathy · Phytochemicals · Novel Weapons Hypothesis · Biochemical Recognition Hypothesis · Leachates

Introduction

Seeds have various adaptations that allow them to detect suitable conditions for establishment, e.g. being able to gauge cues like temperature and moisture (Blossey et al. 2017; Venable and Brown 1988). Further, in some cases, timing of germination can result as a response to chemicals released by conspecifics or other species (Tielbörger and Prasse 2009; Yamawo and Mukai 2017). Such mechanisms can emerge to avoid competition (Preston and Baldwin 1999) or when survivorship depends on the presence of facilitating or nursing species (Lortie and Turkington 2002). It is therefore expected that the release of novel chemicals by alien plants may influence the recruitment of naïve native plants (Callaway and Ridenour 2004; Rabotnov 1982), especially at early ontogenetic stages like germination or seedling growth.

The Novel Weapons Hypothesis (NWH) postulates that specific phytochemicals with harmful effects, so called allelochemicals, can facilitate plant invasiveness by disrupting the metabolism, and thus performance, of evolutionary naïve neighboring plants (i.e. allelopathy sensu Callaway and Ridenour 2004; Inderjit et al. 2011). Allelochemical effects can result from direct plant–plant interactions or indirect interactions with secondary compounds following degradation or transformation by soil biota such as microbes (Inderjit and van der Putten 2010; Inderjit and Weiner 2001). Numerous studies have found evidence supporting the NWH for invasive plants, e.g. *Carpobrotus edulis* (Novoa et al. 2012), *Centaurea* spp. (Callaway and Aschehoug 2000; Callaway and Ridenour 2004; Thorpe et al. 2009), *Phragmites australis* (Rudrappa et al. 2007) and *Eucalyptus globulus* (Becerra et al. 2018). Despite this growing body of evidence, using methods that realistically mimic allelochemical concentrations and interactions under field conditions remains a challenge (Alford et al. 2007).

In their native ranges, plant responses to phytochemicals should be tied to adaptation among species sharing evolutionary experience, potentially shaping their coexistence. In this regard, the Biochemical Recognition Hypothesis (BRH) postulates that, through the recognition of phytochemicals released by potential competitors, plants can identify best conditions for establishment and time their germination accordingly (Renne et al. 2004, 2014). In this context, the BRH argues that the inhibition of germination is not always explained by the exposure to toxic metabolites (i.e. allelochemicals). Instead, the BRH is an adaptive response to a broad range of chemicals released by interacting plants that may aid to avoid strong inter- or intra-specific competition and maximize establishment potential. For instance, previous studies have found decreased emergence of grassland species when exposed to leachates of sympatric species or conspecifics (Renne et al. 2004, 2014), hinting towards a mechanism of competition avoidance. In non-native ranges, the mechanisms underlying the BRH may also apply to co-occurring invasive species that share the same historical native range, a phenomenon that has not yet been explored in invasion biology. Furthermore, closely related species may develop similar responses to the release of phytochemicals if they have very similar niches (Renne et al. 2014), or if not closely related, by having similar eco-evolutionary experiences (Saul et al. 2013). While both the NWH and BRH focus on plant-based chemicals reducing germination, like others, we argue that chemical recognition of some species could also adaptively enhance emergence of other species (Renne et al. 2014). That is, a 'stimulatory' response to phytochemicals could result from the presence of plants, such as nursing or mutualistic species (Bouwmeester et al. 2003; Plakhine et al. 2009; Lortie and Turkington 2002), that might create

the appropriate abiotic conditions for another one to emerge.

Invasive Australian acacias (genus *Acacia*, Fabaceae) represent an interesting study group for assessing con- and heterospecific phytochemical effects, since invaded areas typically consist of dense stands of multiple invasive species (including different acacias) and a few interspersed natives (e.g. Le Roux et al. 2018). Because acacia invasions are often characterized by drastic native biodiversity declines, especially in Mediterranean-type biodiversity hotspots (Le Maitre et al. 2011; Richardson et al. 2011), it remains a research priority to understand the mechanisms that underlie their invasion success. While there have been no generalizations to explain the invasiveness of acacias (Gibson et al. 2011), allelopathy is thought to be important (Aguilera et al. 2015b; Hussain et al. 2011; Lorenzo et al. 2010, 2011). In particular, the potential allelopathy of *Acacia dealbata* Link has been extensively studied using aqueous and natural leachates in Spain and Chile. These studies found, leachates negatively impact germination, seedling development, metabolism, and productivity of native and/or model species (Aguilera et al. 2015a; Carballeira and Reigosa 1999; Lorenzo et al. 2011). Detrimental effects have also been found from aqueous extracts of other acacias such as *A. saligna* (Labill) H.L. Wendl., *A. longifolia* (Andr.) Willd. and *A. melanoxylon* R. Br. (Abd El-Gawad and El-Amier 2015; González et al. 1995; Souto et al. 2001). Arguably, the techniques used to extract the phytochemicals do not necessarily portray in situ field conditions, or make results comparable among different studies or areas. Further, to date, nothing is known about the effects of phytochemicals released by acacias on congeneric species, or how this may impact invasiveness in different ranges using comparable leachate collection methods (but see Lorenzo et al. 2010).

In this paper, we explored whether different Australian acacias have negative effects on naïve species (native species and historically allopatric acacias; following the NWH) and whether they can modulate early ontogenetic processes (germination and early growth) of historically sympatric acacias through phytochemical recognition (BRH). To this end, we assessed the early response of native and acacia species using leachates collected at sites invaded by a number of acacias ('invaded' leachates) and in nearby areas where acacias were not present ('uninvaded' leachates) in two introduced ranges (South Africa and Spain). In support of the NWH, we predicted that allelochemicals produced by invasive acacias would have negative effects on the germination and early growth of naïve species in both countries (Fig. 1). Under the BRH, we hypothesized that acacia-released phytochemicals would curb and/or stimulate early ontogenetic processes of conspecifics or historically sympatric congenerics (Fig. 1).

Materials and methods

Study areas and species selection

The two study areas, the Cape Floristic Region in South Africa and Galicia in northern Spain, share important characteristics: both regions support shrubland vegetation (Basanta and Vizcaino 1989; Cowling and Richardson 1995) and are invaded by Australian acacias (Souza-Alonso et al. 2017; van Wilgen et al. 2011). In both areas, acacia invasions result in increased biomass, litter deposition, and soil nitrogen (Souza-Alonso et al. 2017; Yelenik et al. 2004). However, the native vegetation structure differs between these areas, while in South Africa we have the unique fynbos vegetation of the Cape Floristic Region (Cowling and Richardson 1995), northern Spain is characterized by oak forest along with Atlantic shrubland (Basanta and Vizcaino 1989).

We selected six of the most invasive acacias in the Cape Floristic Region and northern Spain (Richardson and Rejmánek 2011). These included *Acacia cyclops* A. Cunn. ex G. Don, *A. dealbata* Link, *A. elata* A. Cunn. ex Benth., *A. mearnsii* De Wild., and *A. saligna* (Labill) H.L. Wendl. in South Africa, and *A. dealbata*, *A. mearnsii* and *A. melanoxylon* R. Br. in Spain. While many of these acacias co-occur in their invaded areas (e.g. Le Roux et al. 2018), they do not all share overlapping native distributions in Australia. For example, *A. cyclops* and *A. saligna* are native to South Western Australia, while *A. dealbata*, *A. elata, A. mearnsii* and *A. melanoxylon* are native to Southeastern Australia (described in Flora of Australia Online at www.ausflora.org.au).

In both countries we included *Lactuca sativa* (lettuce; Asteraceae) as a test species, since it is

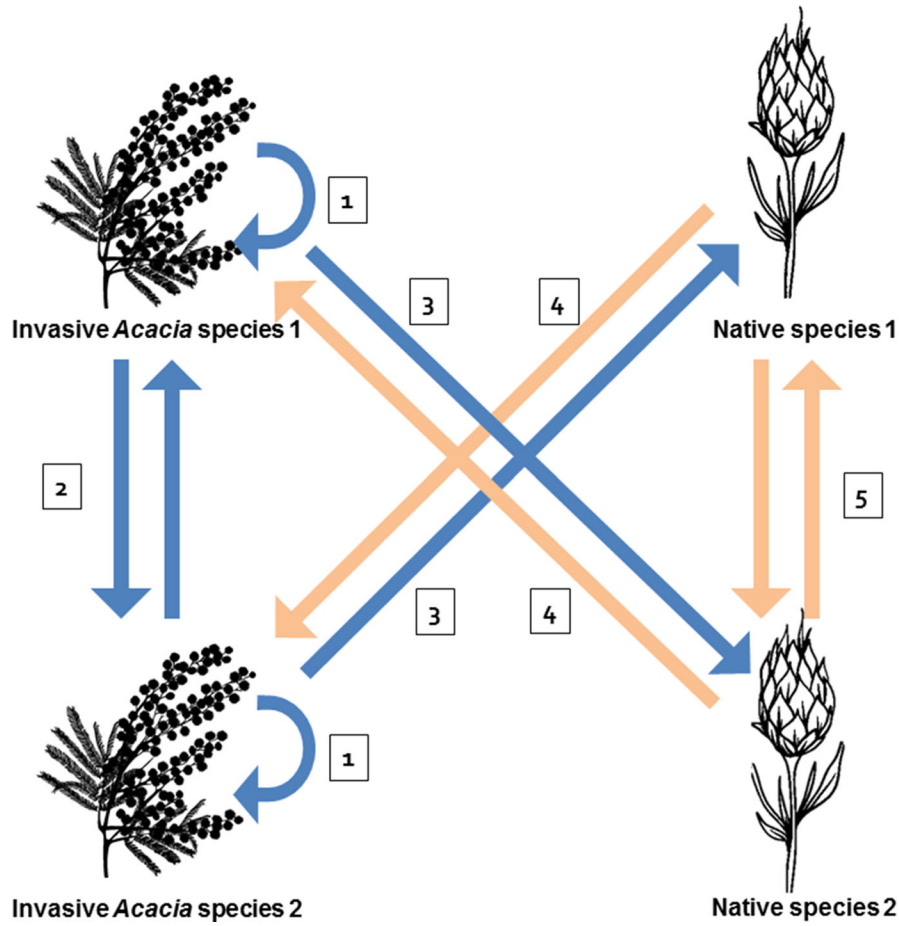

Fig. 1 Schematic representation of the two hypotheses tested in the current study and different possible Scenarios (numbered boxes). Colors of arrows correspond to the terminology used in the main text, where blue arrows indicate effects from leachates collected under invasive acacia species (invaded treatments), and peach-colored arrows leachates collected under native species (uninvaded treatments). Positive and negative responses to leachates under Scenario 1 support an intraspecific biochemical recognition following the Biochemical Recognition Hypothesis (BRH), while positive and negative responses under Scenario 2 support interspecific biochemical recognition (BRH) or, depending on native biogeography, the Novel Weapons Hypothesis (NWH, negative responses only). Negative responses of native species under Scenario 3 support NWH. Any response under Scenario 4 may indicate interspecific biochemical recognition (BRH). Responses (negative or positive) of natives to uninvaded leachates under scenarios 5 likely represent interspecific biochemical recognition (BRH)

considered as a comparable indicator of allelopathy between studies given its fast germination and frequent inclusion in phytotoxic studies (Macías et al. 2000). In South Africa, we used the native species *Vachellia karroo* (Hayne) Banfi & Galasso (formerly *Acacia karroo*, Fabaceae) and *Protea repens* (L.) L (Proteaceae). *Vachellia karroo* is widely distributed throughout South Africa. We selected this species because of its phylogenetic relatedness to Australian acacias (Kyalangalilwa et al. 2013). *Protea repens* is a widespread fynbos shrub occurring in lowland fynbos where acacias are often invasive (Witkowski 1991). In Spain, we chose two natives, *Cytisus striatus* (Hill) Rothm. (Fabaceae) and *Plantago lanceolata* L. (Plantaginaceae). *Cytisus striatus* is a legume shrub common in native Oak forest and shrublands of Galicia in northern Spain, often threatened by *A. dealbata* invasions (Lorenzo et al. 2012; Rodríguez et al. 2017). *Plantago lanceolata* is a forb commonly occurring in disturbed areas in Galicia, which can be found in native shrublands dominated by *Ulex* and *Erica* spp., where invasive *Acacia melanoxylon* and *A. mearnsii* also frequently occur.

Leachate collection

To collect leachates, we identified plots invaded by selected acacias in close proximity to plots where no acacias were present (at least 10 m away; hereafter referred to as 'uninvaded'). We collected two types of leachates for each acacia species: one directly under the selected species' canopy (invaded) and one under the neighboring vegetation in the uninvaded area. In South Africa, the sampling area for *A. mearnsii* and *A. saligna* was the same, so we selected uninvaded plots for both species under native fynbos vegetation. For *A. dealbata*, *A. elata* and *A. cyclops* we chose uninvaded plots in disturbed roadside areas close to acacia-invaded sites. In Spain, uninvaded sites for *A. dealbata* were located in a nearby native oak forest, and those for *A. melanoxylon* and *A. mearnsii* in a surrounding native shrubland.

Leachates were obtained during the flowering season of acacias, which were in June–August 2016 in South Africa and February–April 2017 in Spain. In both countries, we collected leachates under natural rain conditions for *A. dealbata*, but due to a severe drought in South Africa's Western Cape region in 2016, we used distilled water to mimic precipitation of an average rainy July day for all other species. For this, we randomly placed four plastic trays ($23 \times 17 \times 5$ cm) covered with a fine mesh ($\sim$ 1 mm) under the vegetation in invaded and uninvaded areas. We then covered the trays with a few centimeters of soil and litter. Then depending on the leachate type, we either left the trays under the plants for 24 h following a rain event, or covered them with $\sim$ 7 cm of the selected species' leaves and flowers, and subsequently water the trays with distilled water (600 mL of distilled water; $\sim$ 21.45 L rain m^{-2} tray area). We collected the leachates accumulated in trays and transferred them to clean plastic bottles that were immediately stored at $-$ 18 °C (Lorenzo et al. 2011). Leachates were later thawed and filtered using filter paper and preserved in the freezer again until further use.

Seed collection and treatment

All seed material was obtained in 2017. In South Africa, we collected seeds of *A. elata* and *A. saligna* in the field (Table S1) and obtained seed for all other acacias from the Agricultural Research Council's Plant Protection Research Institute (ARC-PPRI; Stellenbosch, South Africa). We bought seeds of native South African species from a local seed supplier (Silverhill Seeds, Cape Town) and *L. sativa* from a local agricultural shop. In Spain, we obtained seeds of *A. mearnsii* and *L. sativa* from local seed suppliers (Company Seeds shop and local agricultural shop), while seeds for all other species were collected in the field (Table S1).

At the start of the experiment, we scarified all acacia seeds by placing them in boiled distilled water for 10 min and then letting them dry, with the exception of *A. melanoxylon* seeds that were scarified using a handheld rotary tool Ryobi® HT20VS equipped with a sanding shank accessory (13×13 mm, 80-grit sanding band, input power 100 W, no-load speed 6000 rpm) for 5 min (Pedrol et al. 2018). *Cytisus striatus* seeds were scarified using the same approach as for *A. melanoxylon*. *Protea repens* seeds need fire cues for germination, we thus soaked them in a smoke-based primer for eight hours (Cape seed and book suppliers, South Africa).

Experimental design

We performed three experiments in 2017. In each country, a germination experiment was set-up in growth chambers with a night temperature of 10 °C (dark) for 14 h and a day temperature of 20 °C (light) for 10 h. An additional glasshouse experiment was setup to assess the effects of leachates on the early growth of South African species during March–April. Experiments had a two-way factorial design, including the two factors 'recipient species identity' (i.e. species being exposed to the leachate) and 'leachate treatment'.

In South Africa, the 'recipient species identity' treatment had eight levels (all invasive and native species), the 'leachate treatment' consisted of 11 leachates (all invaded and uninvaded plus a control consisting of only distilled water). All treatments were replicated five times, making up a total of 440 combinations. We germinated 25 seeds of most species per Petri dishes; except of *A. mearnsii* (20 seeds), *A. elata* (15 seeds) and *P. repens* (20 seeds), due to seed or space availability. Each dish was lined with two layers of Whatman filter paper Grade 6 (90 mm diameter) before adding seeds and received 3 mL of the treatment-specific leachate at the

beginning of the experiment. In order to avoid contamination, we immediately changed the filter paper when any incipient fungal contamination was observed. We put all material inside sealed bags to avoid evaporation and randomly placed them in a growth chamber. Seeds were kept moist during the experiment by adding 1 mL of the respective leachate when necessary. Once all test species first germinated, we took one germinated seed from each treatment combination and replicate ($n = 5$) to investigate the effects on early growth kinetics of the recipient species in the glasshouse. For this, we grew seedlings in polystyrene seedling trays (3 × 3 × 6 cm) filled with sterile silica sand. Each seedling was initially watered with 3 mL of a treatment-specific leachate (invaded, uninvaded and control) followed by 2 mL of the same treatment-specific leachate twice a week.

In Spain, the 'recipient species identity' treatment consisted in six levels and the 'leachate treatment' of seven levels (including control with only distilled water), with five replicates for a total of 210 treatment combinations. In order to avoid fungal contamination, we sterilized all seeds with 1% sodium hypochlorite for 10 min followed by rinsing with sterile distilled water prior to scarification. The same number of seeds were used as for the South African experiment (25 seeds), except for *A. mearnsii*, for which we used 20 seeds per dish due to low seed availability. We initially added 5 mL of the corresponding leachate and subsequently sealed the Petri dishes with Parafilm to prevent evaporation. All plates were randomized daily.

Measurements and statistical analyses

In the South African germination experiment, we counted and removed each seed that had germinated weekly for up to 4 weeks. For the glasshouse study, we allowed seedlings to grow for 3 weeks, harvested the whole plant and later separated roots and shoots in paper bags. This material was subsequently oven-dried at 50 °C for 72 h and later weighed. In Spain, we terminated the experiment when cotyledons of seedlings reached the lids of Petri dishes by placing the plates in a freezer to halt seedling growth (Lorenzo et al. 2010); after 23 days for *A. dealbata*, 28 days for *A. melanoxylon*, 27 days for *A. mearnsii*, 18 days for *C. striatus*, 13 days for *P. lanceolata* and 10 days for *L. sativa*. We subsequently counted the total number of germinated seeds per dish.

In order to test our hypotheses, we separately evaluated the effects of the leachates collected for each invasive species (i.e. invaded, uninvaded and distilled water control) on each recipient species individually. Given that our data had skewed distributions, we compared total germination percentages among leachate treatments by means of generalized linear models with binomial distribution. We tested each model for equidispersion and, when not complying with this assumption, we used a quasibinomial distribution corrected for the dispersion parameter (Crawley 2012). Data from the South African glasshouse experiment followed the assumptions of normality and homogeneity of variances, so we explored the effects of leachates on the performance of each species (dry root and shoot biomass) using ANOVA and compared the treatments using the Tukey test. All statistical analyses were done in the R statistical environment (version 3.5.0) (R Development Core Team 2018).

Results

In South Africa, we found various leachates to affect the early kinetics of invasive *A. dealbata*, *A. elata*, *A. saligna*, and the native *V. karroo* (Table S2 and S3, supplementary information), with no apparent effects on the other species (*A. cyclops, A. mearnsii,* lettuce and *P. repens*). For Spain, we only found treatments to affect *A. dealbata* with no significant effects on other species (Table S4). Specifically, compared to the control treatment, *A. dealbata* showed a mean increased germination of 13% under the *A. dealbata* rain-based uninvaded leachate, followed by the invaded (+8%; GLM, $P_{\text{control}} = 0.02$) in Spain. In South Africa we found *A. dealbata,* when exposed to *A. elata* leachates, to have decreased germination in both invaded (− 4%) and uninvaded treatments (− 8%) compared to the control with pure distilled water (Fig. 2; GLM, $P_{\text{control}} = 0.03$). *Acacia elata* had a mean increase in germination with respect to the control, when exposed to its own leachates (+ 12%) and those collected from *A. mearnsii* (+ 29%), with no significant differences with the uninvaded treatments (Fig. 2*; A. elata,* $P_{\text{invaded}} = 0.01$; *A. mearnsii* GLM, $P_{\text{invaded}} = 0.02$). Relative to the control and invaded treatments (Fig. 2), the germination of *A. saligna* was, on average, higher in uninvaded

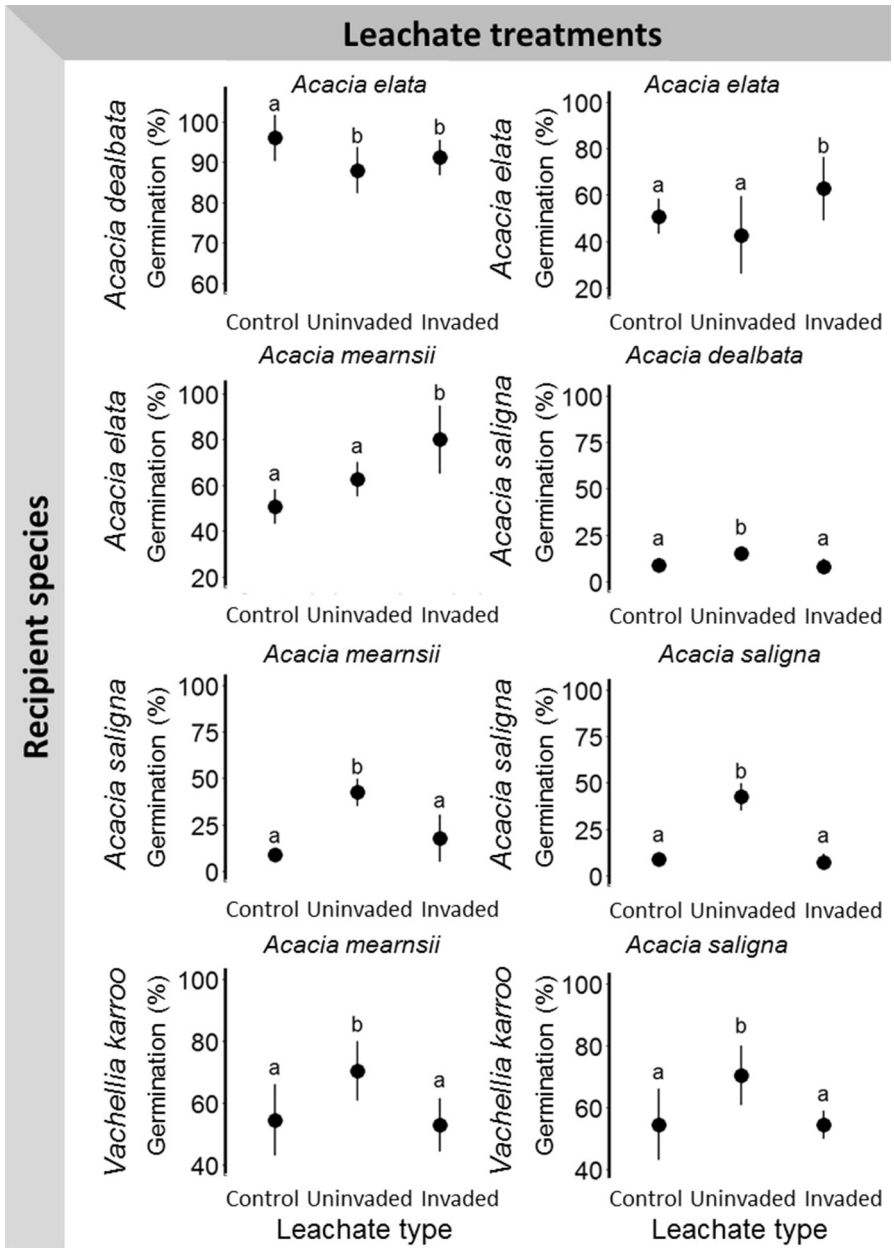

Fig. 2 Results GLMs indicating significant differences in germination (%) among the treatments for invasive and native species ($P < 0.05$) in South Africa; species that did not yield significant differences are not shown. Treatments consisted of pure distilled water (control) and leachates collected in invaded and uninvaded areas. Mean ± SD are shown; different letters indicate significant differences ($P < 0.05$)

treatments collected from *A. dealbata* (6% higher, $P_{control} = 0.03$; 7% higher, $P_{invaded} = 0.01$), *A. mearnsii* (33% higher than control and 13% higher than invaded, $P < 0.01$) and *A. saligna* (33% higher than control and 35% higher than invaded, $P < 0.01$). Compared to the control and invaded leachates, the native South African *V. karroo* showed an average increase in germination when exposed to leachates collected in uninvaded areas near *A. mearnsii* (Fig. 2; 16% higher than control and 17% higher than invaded, $P < 0.1$) and *A. saligna* (Fig. 2; 16% higher than control and 17% higher than invaded, $P < 0.1$).

For the glasshouse experiment, not enough replicates were available for *A. cyclops*, *A. mearnsii* and *V. karroo* shoot/root biomass (due to mortality) under the *A. mearnsii* and *A. saligna* treatments, so these were removed from all subsequent analyses. *Acacia dealbata* seedlings produced significantly higher shoot and

root biomass under the invaded *A. cyclops* leachate compared to the uninvaded treatment, with intermediate shoot biomass values in the control (Fig. 3; ANOVA: cyclops, shoot $F = 6.76$, $P = 0.01$; root $F = 4.63$, $P = 0.03$). Similarly, *Acacia elata* had increased root biomass when exposed to *A. mearnsii* leachates, followed by the control and then the uninvaded treatment (Fig. 3; ANOVA: $F = 4.93$, $P = 0.04$). *Acacia saligna* showed more investment in root biomass under *A. dealbata* and *A. saligna* leachate treatments (Fig. 3; ANOVA: dealbata $F = 4.14$, $P = 0.049$, saligna $F = 22.93$, $P < 0.001$), with intermediate values in the control leachate for *A. dealbata*.

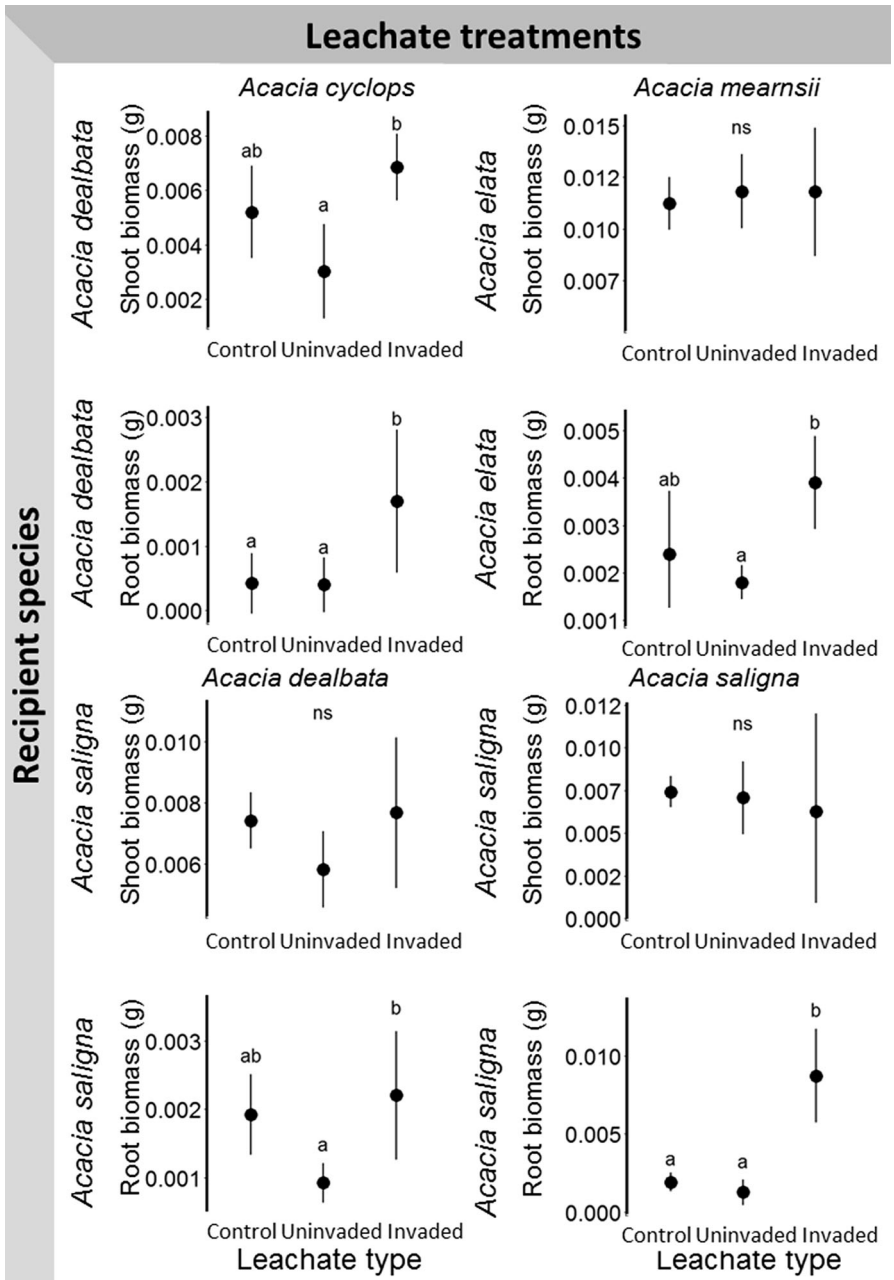

Fig. 3 Results for recipient species differences in aboveground shoot (upper section) and belowground root (lower section) biomass in South Africa. Recipient species that did not yield significant differences in biomass are not shown. Treatments consisted of pure distilled water (control) and leachates collected in invaded and uninvaded areas. Mean ± SD are shown. Significance of treatments effects were tested using ANOVA and post hoc Tukey tests, different letters indicate significant differences ($P < 0.05$) and non-significant differences are indicated by 'ns' ($P > 0.05$)

Discussion

Leachate effects on the germination and growth of recipient species

Our study provides evidence that phytochemicals of native and invasive species affect early ontogenetic processes of both groups. As predicted, we found instances in support of the Biochemical Recognition Hypothesis (Fig. 1), whereby leachates collected under the same or a different species had stimulatory effects on germination and/or early development. Leachate responses were country-, species- and development stage-specific. In other words, we did not find a certain leachate to have the same effect in both countries or on all species tested, and in some instances the observed effects on germination were not evident during early growth. Further, our data did not support the Novel Weapons Hypothesis since we did not find any inhibitory effects of allelochemicals on naïve species (Fig. 1; both native species and historically allopatric acacias).

Almost all significant leachate effects were found in South Africa, even though we had species in common between the two countries (i.e. *Acacia dealbata* and *A. mearnsii*). In Spain, *A. dealbata* was the only species that responded to leachate treatments, whereby less seeds germinated in distilled water compared to treatments involving leachates collected under natural conditions for this species. Despite our findings, previous research from Spain has found evidence for both inhibiting and facilitative effects for *A. dealbata* when exposed to intraspecific compounds or leachates, supporting a mechanism for intraspecific biochemical recognition. Souza-Alonso et al. (2014) showed volatile organic compounds released by the flowers of *A. dealbata* to reduce the species' germination and seedling growth. However, Lorenzo et al. (2010) found invaded leachates to have an auto-stimulatory influence on radicle length using similar methods as we did in our current study. Still, some of our results on other acacias were in line with the latter study, where auto-stimulatory effects resulted in higher germination of *A. elata* and more root growth of *A. saligna* in South Africa. Discrepancies in results between countries and studies are expected to emerge, given the dependency of variation in phytochemical effects on biotic and abiotic conditions (Reigosa et al. 1999). For instance, soil bacteria of different areas can activate or deactivate compounds released by plants differently (Inderjit and van der Putten 2010) and environmental conditions such as temperature and humidity can modulate these effects (Reigosa et al. 1999).

We found evidence for the BRH between acacias (i.e. interspecific BRH) when exposed to leachates collected from sympatric and allopatric congenerics (with respect to the native range in Australia). Specifically, while *A. elata* displayed increased germination and growth when exposed to the sympatric *A. mearnsii* invaded leachate, *A. dealbata* and *A. saligna* showed increased growth under the invaded leachates from the allopatric *A. cyclops* and *A. dealbata* respectively. Renne et al. (2014) also found the emergence of grassland species to be affected when treated with inter-specific leachates of sympatric species, in Argentina and North America, though here emergence decreased. These results may reflect a regulatory mechanism whereby species delay or promote early ontogenetic processes to coincide with suitable environmental conditions. Even though invaded sites often have more than one acacia species (e.g. Le Roux et al. 2018), phytochemical effects between congeners have not been tested before for these species. Doing so is important, given that closely related species share similar evolutionary experiences and thus might have comparable responses to biochemicals produced by, for example, conspecifics (i.e. phylogenetic biochemical recognition; Renne et al. 2014). In other words, regardless of their origin, *Acacia* spp. would be more likely to have similar phytochemistries than phylogenetically distant species, which could influence their invasion success in the introduced range.

We found responses from naïve species to uninvaded leachates, consistent with an interspecific biochemical recognition mechanism (BRH; Fig. 1). Namely, leachates collected under native fynbos species promoted the germination of native *V. karroo* and invasive *A. saligna* in South Africa. Since we did not collect the uninvaded treatments under a specific species in South Africa, such responses cannot be attributed to a particular native species. Further, even though *V. karroo* is native to South Africa, its natural range does not include our study area (Taylor and Barker 2012), thus making it a 'naïve' species in terms of fynbos vegetation. We can, however, assume that the increased germination of *A. saligna* in

uninvaded leachates will aid this species in colonizing new areas.

Significant leachate effects were not observed across all species and, with the exception of *A. elata* under *A. mearnsii* leachates, the responses of the test species were not consistent across the germination and early growth experiments. That is, in some cases we found an effect on germination that was not detected during growth. For example, for *A. saligna*, we found higher germination rates in uninvaded treatments, but enhanced growth when exposed to invaded leachates. These seemingly opposing results can be related to differences in physiological responses of seeds and seedlings to certain compounds in the leachates. Similarly, Souza-Alonso et al. (2014) found recipient species responses to volatile organic compounds (VOCs) released by *A. dealbata,* not only to be species-dependent, but also growth stage-dependent. Similar to our study, but with the opposite effect, some recipient species had no significant germination differences between control and VOCs treatments, and they showed a dramatic reduction in radicle growth later on (Souza-Alonso et al. 2014).

Contrary to our results, previous studies using leachates mimicking natural concentrations and methods similar to ours, found *A. dealbata* leachates to rather have inhibitory effects on early ontogenetic processes of heterospecific naïve species (Carballeira and Reigosa 1999; Lorenzo et al. 2010), which would support the NWH. This inconsistency has previously been ascribed to variation in phytochemical toxicity according to the identity and concentration of substances present in leachates and environmental conditions at the time of their collection (Lorenzo et al. 2010; Reigosa et al. 1999). This highlights the importance of using realistic extraction methods in order to avoid overestimating the allelopathic potentials of species. Even though we did not identify the chemical compounds or their concentrations in our leachates, significant differences between distilled water controls and treatments provide evidence for chemically-induced mechanisms. Elucidating whether our findings reflect the effects of one phytochemical at different concentrations or the interactions of numerous compounds, is an interesting topic to pursue in future research (see Souza-Alonso et al. 2014).

Although we tried to imitate natural conditions during leachate collections, we acknowledge that the interpretation of either a lack of evidence for NWH or support for the BRH in our study should be interpreted cautiously. Indeed, many studies supporting the NWH for the widely studied *A. dealbata,* have utilized extraction methods less comparable to natural conditions, or as in our case, have tested the effects of leachates by germinating seeds on filter paper in the lab (Souza-Alonso et al. 2017). However, under field conditions other factors such as soil invasion legacy effects (e.g. related to alterations in soil chemistry and/or soil microbe communities) or changes in microhabitat seem to override phytochemical-driven effects found for this species in lab essays (Lorenzo et al. 2017; Lorenzo and Rodríguez-Echeverría 2012). Therefore, while our study provides evidence of phytochemical impacts on early ontogenetic processes for a range of acacias, experiments imitating more natural conditions are needed to confirm the phytochemical effects we found here (e.g. using soil collected in the field or common garden methods; da Silva et al. 2017).

Ecological significance of a stimulatory biochemical recognition mechanism

We found support for the Biochemical Recognition Hypothesis, suggesting that chemically-induced signals may facilitate the establishment of acacias in sites that have been already transformed by these species (i.e. positive feedbacks). In line with our findings, a phytochemical facilitative effect as a cue for establishment was previously predicted by Renne et al. (2014), though their experiments only found germination delays as adaptive responses to competitive conditions. The ecological significance of these distinct phytochemical effects could be related to the type of ecosystems studied and thus, the dominating type of species interactions (e.g. facilitation vs. competition). That is, the BRH was previously tested in grassland species, which are highly competitive communities, where an 'avoidance mechanism' might emerge as an advantage for species experiencing low recruitment (da Silva et al. 2017; Renne et al. 2004, 2014). However, in harsh environments where plant communities are mainly structured by the presence of facilitating species, cues received from certain species may indicate the right conditions to germinate or grow (Arroyo et al. 2016; Gross et al. 2013; Lortie and Turkington 2002).

Invasive acacias are known to change soil biota and nutrients loads, leading to positive plant-soil feedbacks (Le Roux et al. 2018; Lorenzo et al. 2013; Rodríguez-Echeverría et al. 2013), and phytochemicals may aid these feedbacks by signaling the right conditions for germination and early growth (Inderjit et al. 2011). Indeed, previous work in both South Africa and the Iberian Peninsula supports the idea of intraspecific facilitative soil effects, whereby early growth kinetics of various acacias respond to previously-invaded soils compared to uninvaded soils (Le Roux et al. 2018; Lorenzo and Rodríguez-Echeverría 2012; Rodríguez-Echeverría et al. 2013). As mentioned, historically allopatric acacias could also react through biochemical recognition if they have similar niches or share similar eco-evolutionary experiences through their similar phytochemistry. Hence, these phytochemically-driven responses may not only aid invasive species in their new ranges, but are most likely also adaptive responses shaping plant interactions in their historical native ranges. Yet, the presence of biochemical recognition as modulator of species interactions, in particular in their non-native ranges, remains to be further explored. Future experiments testing the effects of phytochemicals under field conditions should be carried out to test whether our results hold up for plant-plant interactions under natural conditions (Lorenzo et al. 2017).

Conclusions

In line with the Biochemical Recognition Hypothesis, our study indicates that the release of phytochemicals by native and invasive species can have stimulatory effects on germination and early growth of both groups of species. These responses may be context-dependent, as illustrated here by differences in species responses in South Africa and Spain. The facilitative effects we observed were both intra- and interspecific, whereby leachates collected under invasive acacias stimulated early intraspecific and congeneric ontogenetic processes (of both sympatric and allopatric acacias). Responses among sympatric species are expected due to shared evolutionary experience, but closely related species may also develop biochemical recognition due to niche conservatism or phylogenetic phytochemical similarities. Our results, along with previous studies indicating that acacias change underground conditions to their favor, support the idea that chemically-induced signaling can facilitate the establishment of some acacias species in sites already favorably transformed by other acacias, leading to positive feedbacks. Stimulatory effects of uninvaded leachates on acacias could also potentially aid the invasion process in fynbos ecosystems. In contrast with previous studies, the lack of evidence for the Novel Weapons Hypothesis for acacias could be linked to differences in the environmental conditions, the concentration of chemical compounds, or the species we tested. We also suspect that differences in the type of habitat or community where such chemically-induced mechanisms might emerge would determine whether they will be inhibiting or stimulatory, e.g. whether systems are largely governed by competition or facilitation. Overall, our results show that the effects of leachates are species- and growth stage-specific and can be dependent on the environmental conditions. Future research efforts should focus on unravelling the roles of phytochemicals in plant responses under field conditions to ascertain if similar patterns emerge under natural conditions.

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